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FORMATION OF CHLOROPLAST PIGMENTS AND PHOTOSYNTHETIC POTENTIAL OF CHLOROEMBRYOS IN ANGIOSPERMS

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ABSTRACT

The current information on chlorophyll bearing embryos with special reference to formation of chloroplast pigments and photosynthesis is comprehensively reviewed here. Chloroembryos are reported to contain chloroplasts even at the early stages of embryo development and the formation of chloroplast pigments in majority of chloroembryos is light dependent. Marked changes in form and function of chloroembryos occur when these embryos are allowed to mature in dark. Percentage of radiant energy passing through the fruit wall and seed coat and finally reaching the chloroembryos as determined by using quantum photometer, facilitating the embryos to harvest such a weak and filtered sunlight effectively is reported. The possible role of chloroembryos to photosynthesize *in vivo* using $^{14}CO_2$ fixation and less gorging of assimilates on blocking embryo photosynthesis is also briefly discussed.

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INTRODUCTION

Chlorophyll bearing property of embryos in Angiosperms or its absence is of particular interest because it is not only an embryological but also physiological phenomenon since it may be intimately related to certain peculiarities of plant metabolism which are yet to be elucidated. This review is concerned with critical appraisal of current literature on the physiological studies of chloroembryos. The earlier review by Yakovlev & Zhukova (1980) was on the occurrence and ultrastructure of chloroembryos.

OCCURRENCE OF CHLOROEMBRYOS

Chlorophyll bearing embryos are widely distributed in Angiosperms. The occurrence of green pigments in embryos as well as in endosperm and seed coat of certain flowering plants had already been reported in the middle of last century (Hofmeister, 1858; Flahault, 1879; Lubbock, 1892). The occurrence of green embroys in the families like Anacardiaceae, Celastraceae, Convolvulaceae, Cruciferae, Geraniaceae, Papilionaceae, Plumbaginaceae, Polemoniaceae, Staphyleaceae and Tiliaceae has been reported by Lubbock (1892); Aceraceae, Aponogetonaceae, Araceae, Basellaceae, Basellaceae, Burseraceae, Capparidaceae, Euphorbiaceae, and Scrophulariaceae by Netolitzky (1926); Apocynaceae, Aroideae, Celastraceae, Malvaceae, Meliaceae, Myrtaceae, Leguminosae and Rutaceae by Lubimenko (1906) and Monteverde & Lubimenko (1909). Yakovlev & Zhukova (1975) made a detailed study on the occurrence of green embryos in flowering plants and advocated the division of Angiosperms into leucoembryophytes and chloroembryophytes on the basis of this feature. They found that all the families belonging to Leguminosae were characterized by the green embroys. They considered the embryo as chlorophyll bearing, independent of the fact as to which of the embryo organ contained the pigments and whether the pigments were preserved or not upon seed maturation and dehydration. Kachru & Krishnan (1979) reported the occurrence of green embryos in the mature seeds of *Dendrophthoe falcata*, a Loranthaceae member. The recent review of Yakovlev & Zhukova (1980) reports the occurrence of chlorophyll in embryos from 428 species of Angiosperms belonging to 224 genera and 72 families, out of which 1094 species, 666 genera and 182 families were investigated. Dahlgren (1980) had reported a clear tendency of chloroembryos to occur in non-endospermous seeds besides pointing out some cases, where they lacked chloroembryos. However, Kaladharan (1988) observed endospermous seeds to have maximum amount of chlorophylls than the non-endospermous seeds among 45 species of chloroembryophytes and also observed the tendency of embryos of *Arachis hypogaea* to become green when the developing fruits were brought epigeal.

FORMATION OF CHLOROPLAST PIGMENTS

The chloroplast pigment composition in the green embryos of Angiosperms has been reported by a number of workers (Kaladharan & Vivekanandan, 1983; Palanisamy & Vivekanandan, 1985; Periasamy & Vivekanandan, 1981; Ryczkowski & Szewczyk, 1973; Szewczyk

& Ryczkowski, 1977; Yakovlev & Zhukova, 1973; Zhukova 1963, 1965, 1967, 1972). Ryczkowski & Szewczyk (1973) reported that in *Haemanthus katharianae* the content of chlorophylls *a* and *b* in 52-102 days old embryos increased concomitantly with the growth of the embryo, whereas in 102-118 days old embryos there was no correlation between the content of chlorophylls and the growth of the embryo, and the chlorophyll content in these embryos decreased during this period. In the embryos older than 118 days, chlorophyll content again increased and the chlorophyll a/b ratio during embryo development was inconsistent showing ups and downs. Szewczyk & Ryczkowski (1977) found in *Laburnum anagyroides* and *Caragana* sp. that chlorophyll content per embryo increased concomitantly with the growth upto a certain stage and then dropped, whereas in *Lunaria annua* chlorophylls increased irregularly during development of the embryo.

Periasamy & Vivekanandan (1981) opined that biosynthesis of chlorophyll in the embryos of *Cyamopsis tetragonoloba* was dark mediated and the content of chlorophyll increased during embryo development and declined when the fruit and seeds started drying. They also observed that although chlorophyll in these embryos might be synthesized by a different pathway (non-photosensitized enzymic reduction of protochlorophyllide), they were found to be photosynthetically active similar to those of leaf pigments. However, Kaladharan & Vivekanandan (1983) reported with experimental evidence that chlorophyll bisoynthesis in the embryos of *Cyamopsis tetragonoloba* was light dependent. Chloroembryos of this species were found to have maximum chlorophyll content during 16-18 DAA (days after anthesis) and then gradually declined (Kaladharan, 1988).

ULTRASTRUCTURAL STUDIES

The ultrastructure of plastids in green embryos has been studied by Orsenigo (1964), Rezen-de-Pinto (1964), Bain & Mercer (1966), Zhukova & Yakovlev (1966), Klein & Pollock (1968).Opik (1968),Marin & Dengler (1772),Zhukova(1972,1975), Pinfield et al. (1973),and Yakovlev & Zhukova (1973). These studies have shown that in reality they are chloroplasts,despite a number of features distinguishing them from mesophyll chloroplasts. The most conspicuous difference is the irregulary amoeboid form of the embryo chloroplasts. The numerous invaginations and evaginations enlarge the outer surface. The inner envelope membranes are also invaginated (Zhukova, 1972, 1975). The embryo chloroplasts differ considerably between different taxa. Variation are seen in all the structural elements *viz.*, large peristoms, evaginations free from the membrane systems and in the size and contents of the vesicles (Zhukova, 1975). Sharma (1977) described the plastid differentiation in the developing embryo of *Capsella bursa pastoris* as well as structural changes in chloroplast induced by light and dark. Octant and globular stages of embryos possessed relatively undifferentiated plastids. Chloroplasts in the torpedo-shaped embryos showed little lamellar differentiation but lacked grana formation, whereas in mature green embroys the chloroplasts became fully differentiated with varying number of well developed grana. Treatment in continuous illumination with white light of low intensity completed the transformation of etioplast into

chloroplast. The quiescent chloroembroys of clementine (*Citrus nobilis* x *Citrus aurantium amara pumila*) showed bifacial orientation (Casadoro & Rascio, 1987). They also reported that the plastids of the outer adaxial layer and inner regions develop differently. It is reported that in light the plastids of the outer adaxial layer differentiate into photosynthetically active thylakoid system with an ultrastructural organization and a polypeptide composition resembling that of leaf chloroplasts. The 'inner' chloroplasts maintain an organisation reminescent of the quiescent embryo and never get beyond photosynthesis/respiration compensation point. They suggested that the above differentiation is not strictly photodependent.

DARK SYNTHESIS OF CHLOROPHYLL

The pattern of chlorophyll synthesis in leaves parallels the differentiation of proplastids into functional chloroplasts in light. However, Kirk & Tilney - Basset (1967) reported that several members of gymnosperms, ferns, mosses and algae were able to synthesise chlorophyll in dark. Dark synthesis of chlorophyll was observed in the primitive species of *Metasequoia glyptostroboides* (Laudi & Manzini, 1975). Bogdanovic (1973) stated that biosynthesis of chlorophyll in pine cotyledons in dark was purely an enzymatic process. Schmidt (1924) found that the excised embryos of *Pinus* and *Picea* synthesized chlorophyll in dark, only when they were in contact with living megagametophytic tissue.

It is generally accepted that angiosperms do not make chlorophyll in dark, although certain plants like, *Tradescantia albiflora* (Adamson et al., 1980) *Tradescantia blossefeldiana* (Adamson & Hiller, 1981), wheat (Seybold, 1948), oat seedlings (Goodwin & Owens, 1947) and *Citrus* cotyledons and water lotus seedlings (Meyer et al., 1960) synthesized chlorophyll in dark. Granick (1967) observed dark-chlorophyll formation in *Chlorella*, *Scenedesmus* and certain inner tissues of tomato fruit. Meister (1985) reported that chlorophyll present in dark-germinated cotyledons of *Tetragonolobus purpurens* was obviously carried over by the seed and therefore, is considered to be formed by light prior to germination. However, the leaves and cotyledons of seedlings raised from artificial y etiolated seeds of *Dolichos lablab* developed chlorophylls *a* and *b* and carotenoids in significant amounts which were synthesized *de novo* and that cloroplast pigments were not carried over from the seeds (Palanisamy & Vivekanandan, 1987). Shading the fruits of *Cyamopsis tetragonoloba* with light proof black polythene sheet blocked chlorophyll formation and resulted in etiolation of green embroys and fruit walls and such seeds upon germination in light showed 70% reduction of total chlorophyll on *de novo* synthesis in the primary leaves (Kaladharan, 1988).

EFFECT OF AMITROLE (3-AMINO-1, 2, 4, -TRIAZOLE) ON CHLOROPLAST DEVELOPMENT

Aminotriazole (amitrole), a herbicide and a potent inhibitor of chloroplast development inhibited chlorophyll formation in light-grown plants (Bartels & Weier, 1969; Rudiger & Benz, 1979). Vivekanandan & Gnanam, (1975, a, b, c,) pointed out the importance of light in amitr le– induction of chlorosis with-

out any morphogenetic effect in *Canna edulis*. Therefore, aqueous aminotriazole is being employed to find out whether chloropyll formation is by the normal light dependent pathway (Periasamy & Vivekanandan, 1981; Palanisamy &Vivekanandan, 1985; Kaladharan, 1988; Padmanabhan & Regupathy, 1977).

Spray of aminotriazole on developing fruits of *Cyamopsis tetragonoloba* caused bleaching of fruit wall, while the chloroplast pigments in the embroys of the same fruit were not affected (Periasamy & Vivekanandan, 1981). Padmanabhan & Regupathy (1977) investigated that in *Crotalaria juncea*, amitrole caused chlorosis of fruit wall and seed coat but did not interfere with embryo chlorophyll. They also pointed out that he isolated embryos grown on agar medium containing amitrole were susceptible to bleaching. Palanisamy & Vivekanandan (1985) reported that aminotriazole spray caused chlorosis of not only fruit wall and leaf but also green embryos of *Dolichos lablab*. However, in *Cyamopsis tetragonoloba* amitrole's failure to cause bleaching of embryos may be due to the thick endosperm covering, the embryo allowing little or no amitrole to pass through (Kaladharan, 1988). This is further supported by the finding of Padmanabhan & Regupathy (1977) that amitrole caused bleaching of embryo chlorophyll only when the embryos were isolated and directly grown on agar medium. In *Dolichos lablab*, there is no endosperm layer surrounding the embryo.

EFFECT OF RED AND BLUE RADIATIONS

Phytochrome is well known to initiate and control chloroplast formation and synthesis of many thylakoid components (Lichtenthaler & Buschmann, 1978; Kasemir, 1979). Red light was found effective in arresting loss of chlorophyll, protein, total soluble sugars and reducing sugars in excised rice leaves (Sen et al., 1984). Blue light was found to be more effective than red in accelerating chlorophyll accumulation in *Euglena gracilis* and *Scenedesmus obliqquus* (Senger & Bishop, 1972; Oh-Hama & Senger, 1975; Humbeck et al., 1984), *Chlorella vulgaris* (Kowallik & Schurmann, 1984), and *Sinapis alba* (Wild & Holzapfel, 1980). Blue light is found to be necessary not only for the synthesis of chlorophyll but also for the development of chloroplast as a whole (Sundqvist et al., 1980). Effect of blue light in increasing electron transport capacity (Appleman & Pyfrom, 1955) and size of chloroplasts (Mohr, 1956) are well established in literature. However, information on chloroembryos is rather scanty. Red radiation enhanced the levels of chloroplast pigments in green embryos of *Dolichos lablab* (Palanisamy & Vivekanandan, 1985), and in the greening embryos of *Cyamopsis tetragonoloba* (Kaladharan, 1988).

EFFECT OF LIGHT INTENSITY ON CHLOROPLAST DEVELOPMENT

The morphological development of plants, leaves and of chloroplast largely depends on the light intensity in which they are grown (Boardman et al., 1974; Lichtenthaler, 1979, 1981). Quality and percentage of radiant energy reaching palisade and spongy chloroplasts of bifacial leaves were measured by Terashima & Saeki (1983) and Kulandaivelu et al. (1983) and found that these chloro-

plasts of the same leaf receive different quality and intensity of sunlight. Palisade chloroplasts are adjusted to high light intensity and of spongy chloroplasts to low light intensity (Terashima & Inoue, 1984). Analogous to these reports, chloroplasts of chloroembryos might also adjust to their *in situ* light environment as these green embryos reside deep inside the fruit being covered by fruit wall, seed coat and sometimes by endosperm. Percentage of sunlight passing through fruit wall and seed coat and finally reaching the embryos of *Dolichos lablab* (Palanisamy & Vivekanandan, 1986a) and that of *Cyamopsis tetragonoloba* (Kaladharan, 1988) has been determined using quantum photometer. It is reported that medium to low light intensity (4-6 to 0.33 Wm^{-2}) favours synthesis of maximum chloroplast pigments (Grumbach & Lichtenthaler, Lichtenthaler et al., 1982).

PHOTOSYNHESIS

Developing chloroembryos of *Haemanthus katharinae* (Ryczkowski & Szewczyk, 1973), *Crambe tatarica* (Szewczyk, 1974), *Laburnum anagyroides* & *Lunaria annua* (Ryczkowski & Szewczyk, 1975) and *Dolichos lablab* (Palanisamy & Vivekanandan, 1986b) have been reported to photosynthesize *in vitro*. Photosynthetic rate in young embryos was higher than the older ones on fresh weight basis (Szewczyk, 1974; Ryczkowski & Szewczyk, 1975). Periasamy & Vivekanandan (1981) reported that in the embryos of *Cyamopsis tetragonoloba* apparent photosynthesis and respiration increased during embryo development and that of fruit and fruit wall was too low as compared to the embryo. They also pointed out that the activity of RuBP carboxylase in these embryos was nine times higher than PEP carboxylase. Kachru & Krishnan (1979) have measured in the extracts of green embryos of *Dendrophthoe falcata*, active enzymes of photorespiration such as phosphoglycolate phosphatase, 3 phosphoglycerate phosphatase and NADH linked glyoxylate reductase. Hole & Scott (1981) by shading the fruits of *Pisum sativum* found that shading pea fruits reduced its growth and yield (dry weight) and also opined that prevention of fruit wall photosynthesis leads to reduction in yield. However, no special mention was made about the green embryos and their photosynthesis. Palanisamy & Vivekanandan (1986b) measured the Hill activity and photosynthetic CO_2 uptake in green embryos of *Dolichos lablab* and reported that photosystems I & II are functional and respiratory rate in young embryos was twice as high as photosynthetic rate. Singh (1988) observed higher activity of PEP Case than RuBP Case in pod wall and seed and seed coat of chickpea and opined that these fruiting structures utilized PEP Case for recapturing the respired CO_2. Khanna & Bansal (1988) reported that CO_2 release from the intact pod, pod wall and seed increased, as seed developed and dark respiration rates were higher than in light. Kaladharan (1988) studied the possible role of chlorophpll in *in vivo* photosynthesis of chloroembryos of *Cyamopsis tetragonoloba* $^{14}CO_2$ fixation, fluorescence measurements and photosynthetic CO_2 uptake and thus considered the chloroembryos as partly autotrophs.

BASIC BIOCHEMICAL CONSTITUENTS DURING SEED DEVELOPMENT

Singh et al. (1981) observed that in the developing seeds of chickpea cultivars, the content of soluble sugars, starch, protein-nitrogen and salt soluble proteins gradually increased upto 35 days after flowering and then stablized till maturity, whereas the accumulation of seed protein-amino acids was irregular. They pointed out that most of the biochemical activity apparently occurred between 14-28 days after flowering. Similarly, Palanisamy & Vivekanandan (1986a) found that in the embryos of *Dolichos lablab* soluble proteins, total soluble sugars and soluble starch increased, while free amino acids decreased during development between 10-21 DAA. Starch content increased till maturity, while total soluble sugars increased upto 12 DAA in the seeds of *Pheseolus vulgaris* (Bhambri & Malik, 1982). Kaladharan (1988) investigated the effect of presence or absence of chlorophyll in the embryos on the levels of accumulation of basic biochemical constituents and minerals during seed development in the chloroembryos of *Cyamopsis tetragonoloba*. About 50% reduction in the levels of basic biochemical constutents was observed except total free amino acids when chloroembryos were made etiolated during the peak pod filling stage (16-28 DAA).

CONCLUDING REMARKS

From the perusal of literature, formation of chloroplast pigments in developing embryos of chloroembryophytes is obviously dependent on transmitted light of low intensity through fruit wall, seed coat, etc. However, occurrence of green pigments in the embryos of fruits having hard and stony fruit wall such as *Samadera indica* and *Spondias pinnata*, where there is little or no possibility of light reaching the embryos as well as disappearance of green colour from the embryos upon shading of the fruits is still an enigma.

Because of sufficient radiant energy reaching the embryos and proper concentration of CO_2 inside the fruit, it is likely that the chloroembryos can photosynthesize *in vivo*. Employing micro oxygen electrode without injuring the embryo one might confirm the *in vivo* photosynthesis of chloroembryos. Poor development of the embryos with considerable reduction in the levels of storage products is observed even when the embryos are still attached to the plant but prevented from sunlight (made etiolated) by masking, indicates possible autotrophic nature of chloroembryos and might open up new vistas in the field of physiology of seed development and manipulation of seed quality for further improvement.

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